

Synaptotagmin-11 inhibits clathrin-mediated and bulk endocytosis

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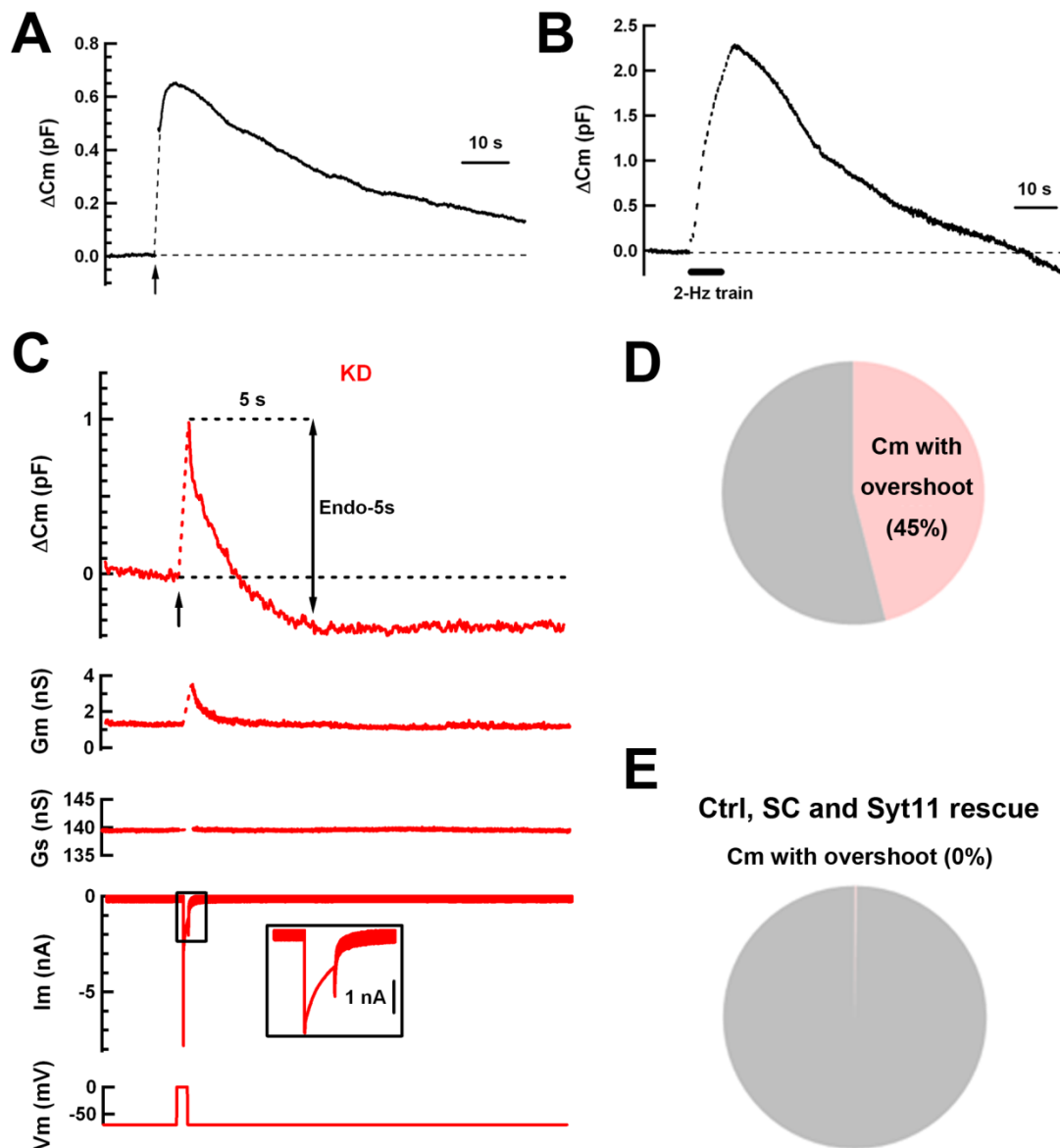
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Appendix Figure S5. Syt11 expression levels in Syt11-KD and rescued DRG neurons.

Appendix Figure S1



Appendix Figure S1. Syt11 knockdown induces endocytosis overshoot in DRG neurons

A, B. Representative membrane capacitance (C_m) traces recorded from a DRG neuron induced by a 200-ms depolarizing pulse (arrow) (A) or a 2-Hz train of twenty 100-ms depolarizing pulses (B).

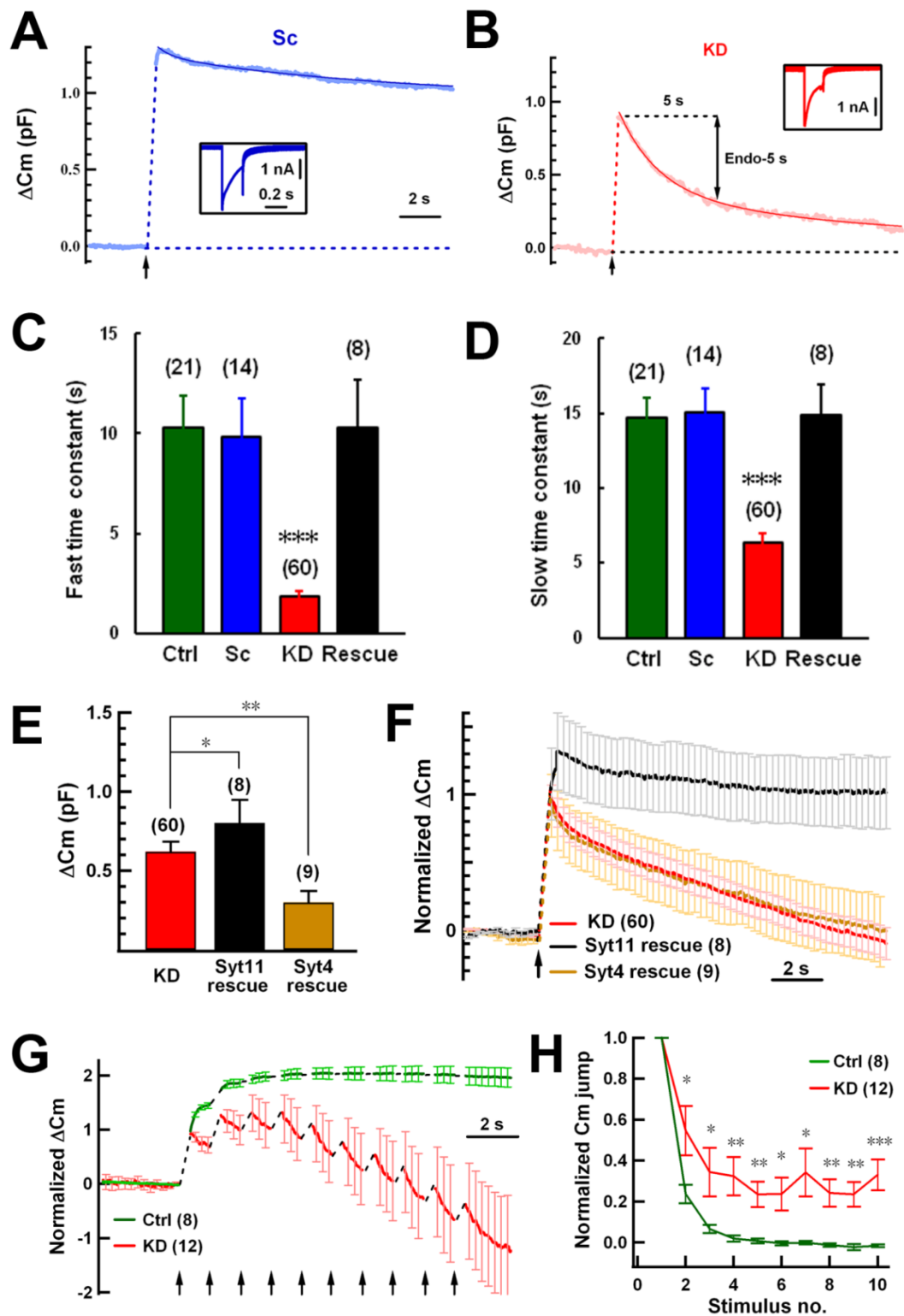
C. Representative C_m trace with endocytosis overshoot recorded from a DRG neuron. Membrane conductance (G_m), series conductance (G_s), membrane current (I_m), and

the voltage clamp protocol (V_m) are shown in the lower traces.

D, E. Percentages of C_m traces with overshoot in Syt11 KD neurons (D, $n = 60$) *versus* control (Ctrl) (E, $n = 21$), scrambled shRNA (SC) (E, $n = 14$), and Syt11 KD neurons with Syt11 rescue (E, $n = 8$).

Data were collected from 4 (Sc, Rescue), 8 (Ctrl), and 16 (KD) independent experiments.

Appendix Figure S2



Appendix Figure S2. Syt11 KD accelerates endocytic C_m decay and vesicle pool replenishment in DRG neurons.

A, B. Representative C_m traces recorded from DRG neurons induced by a 200-ms

depolarizing pulse (arrows). The endocytic C_m decay was fitted to a double-exponential decay function (solid blue and red, fitted curves). DRG neurons were transfected with plasmids expressing shSyt11-2 (Syt11 KD, KD) or scrambled shRNA (Sc) and C_m recording was performed 5 days after transfection. Insets show Ca^{2+} currents recorded in the same neurons.

C, D. Time-constants of the fast and slow phases of endocytosis in Control (Ctrl), Sc, KD, and rescued (with the shSyt11-2-resistant form of Syt11) DRG neurons. Data were collected from 4 (Sc, Rescue), 8 (Ctrl), and 16 (KD) independent experiments.

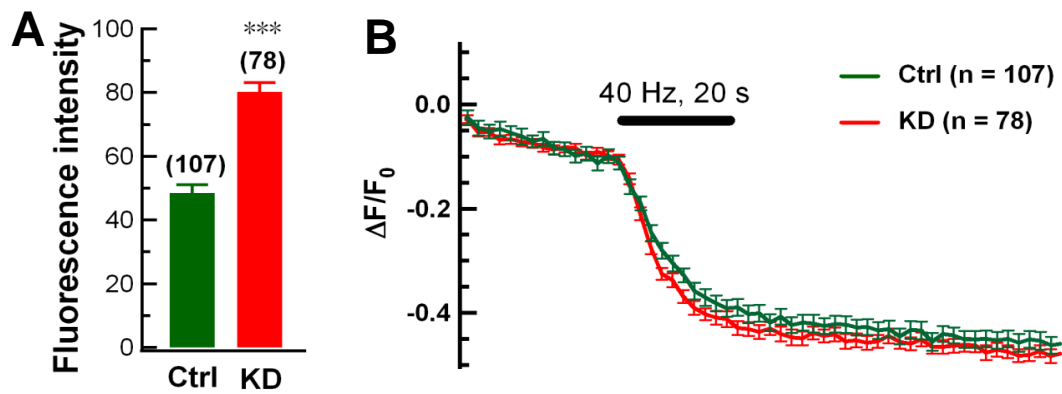
E. Peak C_m changes recorded from Syt11 KD neurons, and KD neurons with Syt4 or Syt11 rescue. Mean \pm s.e.m.; t -test, $*P < 0.05$, $**P < 0.01$.

F. Normalized C_m traces recorded as in (E). Data were collected from 4 (Rescue) and 16 (KD) independent experiments.

G, H. Normalized ΔC_m (A) and C_m jumps (B) induced by a 1 Hz train of 10 pulses (arrows). The normalized C_m jumps were calculated by dividing the ΔC_m values in response to each pulse with that induced by the first one. Data were collected from 3 independent experiments.

All data are presented as mean \pm s.e.m. One-way ANOVA for C-F, Student's t -test for G and H, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Appendix Figure S3



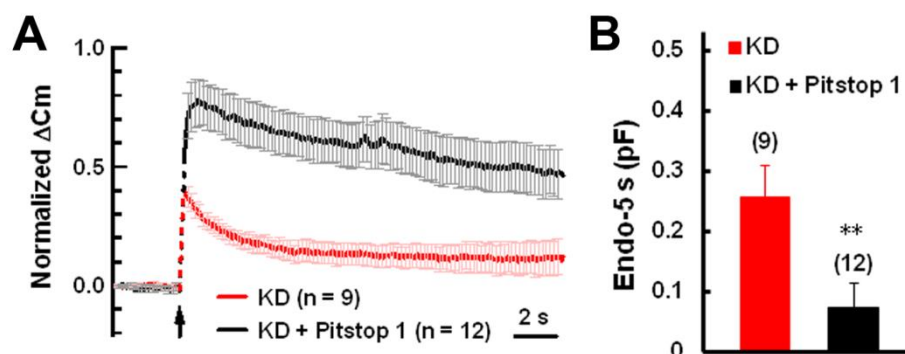
Appendix Figure S3. Syt11 KD increases FM4-64 uptake without affecting exocytosis at hippocampal synapses

A. Fluorescence intensity of FM dye taken up into synaptic boutons. Student's *t*-test, *** $P < 0.001$.

B. Normalized fluorescence intensity ($\Delta F/F_0$) used to assess exocytosis at hippocampal synapses.

Data are presented as mean \pm s.e.m of 3 independent experiments.

Appendix Figure S4



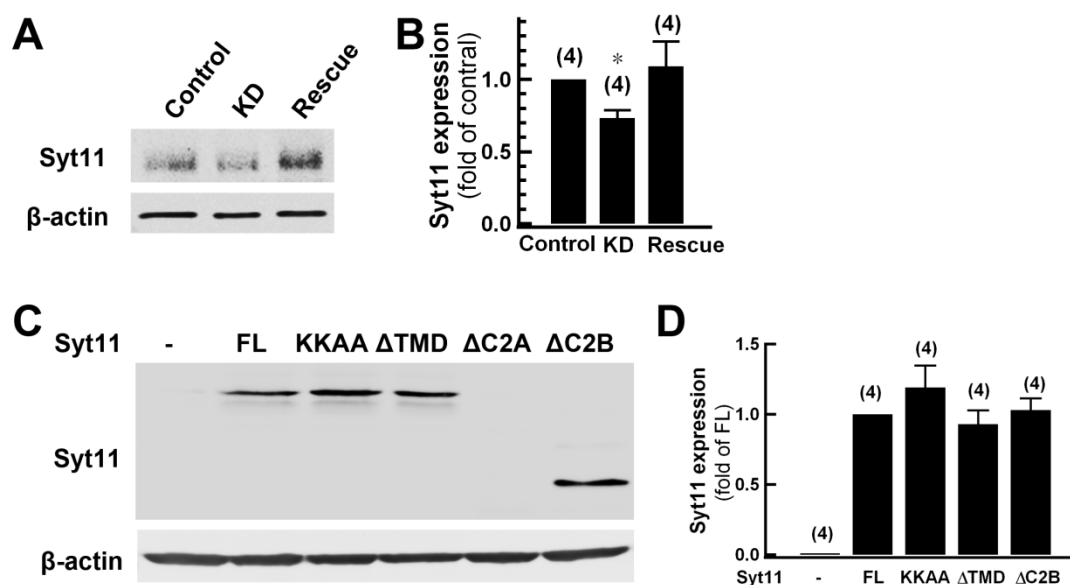
Appendix Figure S4. Syt11 knockdown-induced fast endocytosis is partially blocked by Pitstop 1

A. Averaged ΔC_m traces induced by 200-ms depolarization in the presence and absence of 30 μM Pitstop 1. Endocytic inhibition by Pitstop 1 is shown by the difference in C_m traces recorded from Syt11 KD neurons (KD) and those dialyzed with Pitstop 1 (KD + Pitstop 1).

B. Endo-5s recorded as in (A).

Data are presented as mean \pm s.e.m of 3 independent experiments. Student's *t*-test, $**P < 0.01$.

Appendix Figure S5



Appendix Figure S5. Syt11 expression levels in Syt11-KD and rescued DRG neurons.

A, B. Immunoblotting for Syt11 expression in control (scrambled shRNA), KD (shSyt11-2), and Syt11-rescued DRG neurons. DRG neurons were transfected with plasmids expressing scrambled shRNA, or shSyt11-2 (Syt11 KD, KD) with or without Syt11-rescue and cultured for 5 days. Transfected cells were collected with a fluorescence-activated cell-sorter and the whole-cell lysates were used for immunoblotting analysis. The results revealed similar expression levels of total Syt11 in control and rescued cells (rescued \sim 1.07-fold that in control cells).

C, D. Immunoblotting for the expression of the different Syt11 mutations in HEK2993 cells. HEK2993 cells were transfected with plasmids expressing full-length Syt11 (FL), or Syt11 harboring a deletion of the transmembrane domain (TMD), C2A, or C2B, or the KKAA mutant (replacing the two conserved lysine residues with alanines in the

AP-2-binding site) and immunoblotting was performed ~ 24 h after transfection.

Please note that $\Delta C2A$ was hardly detectable due to deletion of part of the antibody epitope. These experiments ruled out the possible misfolding of mutants after deletion or point mutation, and suggested that most of the rescued constructs expressed at similar levels.

Data are presented as mean \pm s.e.m of 4 independent experiments. One-way ANOVA,

* $P < 0.05$.